PATENTS

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In application of

Christian BELMANT et al.

CONFIRMATION NO. 6944

Serial No. 09/786,055

GROUP 1635

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Examiner R. Schnizer

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FOR THE PRODUCTION THEREOF
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DECLARATION Of Jean-Jacques FOURNIE

Commissioner for Patents

Washington, D.C. 20231

Sir :

I Jean-Jacques Fournié, declare that I am a co-inventor of United States Patent Application No. 09/786,055 and that my technical background and qualifications are as follows:

Jean-Jacques FOURNIE, PhD Biochemistry 1986, HDR 1994, currently research director of the Centre National de la Recherche Scientifique, 55 publications in the field, 2 awards from National Cancer leagues, experienced with 14 years of head of research group working on biochemistry of natural substances at Centre National de la Recherche Scientifique and the Institut Nationale de la Santé et de la Recherche Scientifique.

I have engaged in the study of the effects of the phosphoepoxide compounds on lymphocytes cultured *in vitro* (as reported in the examples disclosed in the application) and *in vivo*. Indeed, I am familiar with the above-identified application and believe that the phosphoepoxide compounds disclosed therein

are effective in activating lymphocytes. In particular, it has been my experience that compounds of formula (2) are particularly effective in activating Tγ9δ2 lymphocytes.

Confirmatory studies of the *in vivo* effects of the phosphoepoxide compounds of Formula (2) described in paragraphs (3 to 5) below clearly demonstrate that use of the phosphoepoxide compounds of formula (2) *in vivo* lead to an expansion/activation of lymphocytes.

In vivo gamma delta cell activation following 5 day intravenous administration of phosphoepoxide compounds of Formula (2) (the compound referred to as EpoxPP) in the cynomolgus monkey was studied. The objective was to study the *in vivo* effect of EpoxPP injected *in vivo* on the activation of gamma delta T cells. As there is no equivalent of Vg9Vd2 cells in rodents, the study was completed in primates.

The study protocol was as follows: Epoxide Pyrophosphate (EpoxPP; IUPAC Name : (R,S) 3,4-epoxy-3-methyl-1-butyl-diphosphate trisodium salt) was synthesized and prepared as concentrated solution of 1mg/ml in water. The total amount of product to be injected was 20 mg, calculated as follows: 5 (number of injection)*0.2mg/kg (dose)*5 (average weight of an animal)*4 (number of animal treated). 4 monkeys were treated with EpoxPP and 4 monkeys were treated with placebo.

Two sets of analyses were carried out:

(a) In a first analysis, the amount of gamma delta cells present in whole blood samples from the animals was determined by a 3 colour (Vd2 FITC, CD69 or CD25 PE, CD3 Pecy5) flow cytometry test. Measurements were made on blood samples taken at 0, 7, 14, 29 and 44 days after injection of EpoxPP. Results of flow cytometry are shown in Graph 1. A clear expansion was observed in monkeys 31 and 32 (EpoxPP group) while no expansion was observed in the placebo group. The greatest expansion was observed at day 29, and the expansion was still observed at day 44.

(b) In a second set of analyse, the capacity of gamma delta cells to proliferate in IL2 was determined, which involved the steps of culturing 1 million ficolled cells for 10 days in the presence of either medium alone, IL2, or IL2 + EpoxPP. Monkey samples were taken at 0, 7, 14, 29 and 44 days after injection of EpoxPP. Flow cytometry experiments were performed at the end of the 10 days, and amounts of gamma delta T cells were determined by flow cytometry. Result of flow cytometry after 10 day expansion in IL2 or IL2 + EpoxPP compound are summarized in the graphs 2 and 3. No evidence of expansion was observed in no group treated with IL2 alone. Significant expansion of relative numbers of delta 2 positive cells were observed in 2 animals in the treated groups (monkeys 31 and 32 in the EpoxPP treated groups). The expansion was observed in the EpoxPP treated group at day 29. The expansion was still observable at day 44 for monkey 32. Significant decrease of relative numbers of delta 2 positive cells was observed at day 7 in both placebo and treated group.

No acute toxicity was observed after injection of EpoxPP injections. Vital signs, rectal temperature, food consumption was not affected by injection of the 2 products at the dose used.

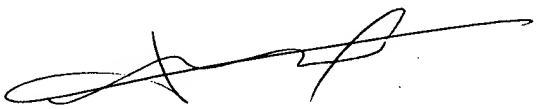
In conclusion a clear expansion/activation was seen in monkeys (animals 31 and 32) in the EpoxPP group. No expansion was seen in the Placebo group. Thus, there is a clear response (expansion of peripheral Vd2+ T cells) in animals treated with EpoxPP as compared to the placebo group.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further these statements are made with the knowledge that wilful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code and that such wilful false statements may jeopardize the validity of the application or any patent issued thereon.

Date

1 Aug 2005

Jean-Jacques FOURNIE



Attachment 1

Figure 1

%Vd2+ on CD3+ cells on macaque lymphocytes : ex-vivo analysis after EpoxPP administration

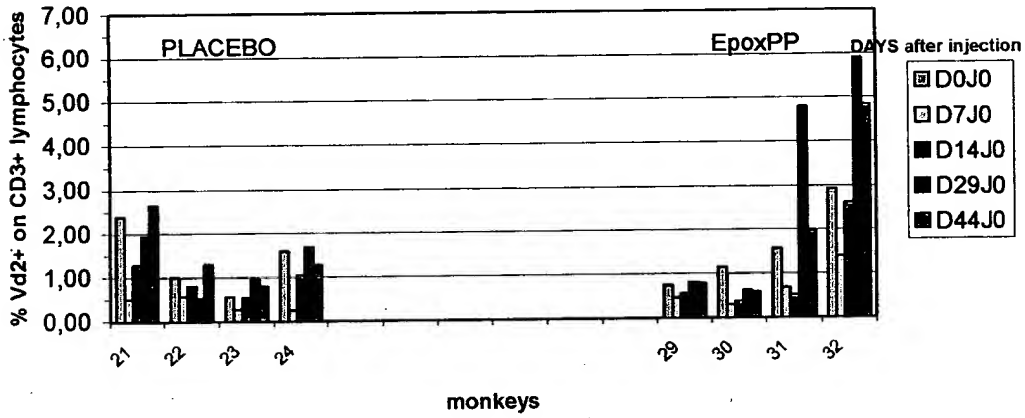
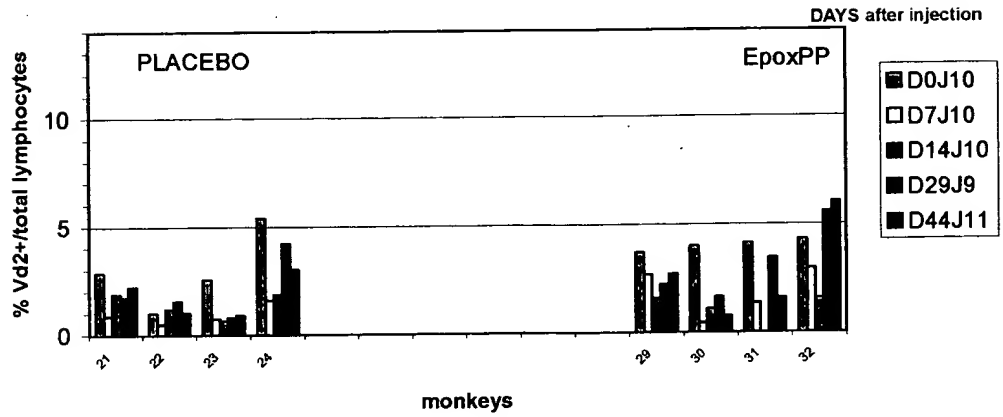


Figure 2

%Vd2+/total macaque lymphocytes after EpoxPP administration: in vitro analysis of about 10 days cultures in IL-2



Attachment 2

Figure 3

%Vd2+/total macaque lymphocytes after EpoxPP administration:
in vitro analysis of about 10 days cultures in IL-2+EpoxPP